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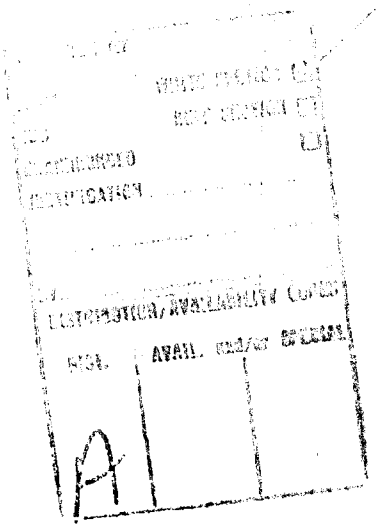
AN EFFECTIVE, READILY AVAILABLE TREATMENT FOR ACUTE RADIATION INJURY IN BEAGLES

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland



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13. ABSTRACT

Generally available clinical methods were used to treat radiation injured beagles. Massive doses of antibiotics and other prophylactic measures were followed by treatment based on clinical judgment; effectiveness of the medical care was judged by the mortality response. The $LD_{50/60}$ of beagles receiving treatment was 276 rads of mixed gamma-neutron radiation at 17 rads/minute; the $LD_{50/60}$ for similarly irradiated beagles receiving no medical treatment was 218 rads. Survival time, symptomatic, hematologic, pathologic, and bacteriologic comparisons were also made. An additional group of irradiated beagles received similar medical care plus intravenously administered leukocyte and platelet concentrates; these dogs responded favorably to treatment following doses as high as 330 rads.

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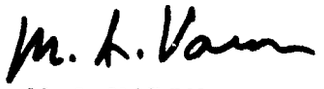

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ABSTRACT

Generally available clinical methods were used to treat radiation injured beagles. Massive doses of antibiotics and other prophylactic measures were followed by treatment based on clinical judgment; effectiveness of the medical care was judged by the mortality response. The $LD_{50/60}$ of beagles receiving treatment was 276 rads of mixed gamma-neutron radiation at 17 rads/minute; the $LD_{50/60}$ for similarly irradiated beagles receiving no medical treatment was 218 rads. Survival time, symptomatic, hematologic, pathologic, and bacteriologic comparisons were also made. An additional group of irradiated beagles received similar medical care plus intravenously administered leukocyte and platelet concentrates; these dogs responded favorably to treatment following doses as high as 330 rads.

I. INTRODUCTION

A more optimum treatment regimen for radiation injured personnel is a continuing goal of radiobiology research. Treatment of the hematopoietic syndrome has been studied in several animal species. Neutropenia and thrombopenia are the causes of poor prognosis in this syndrome.[4,15] Antibiotic therapy reduced radiation mortality in rodents.[8,11,16] Antibiotic treatment of dogs following radiation doses in the median lethal range was reported previously to be of limited value.[3,7,9] Some control of postirradiation hemorrhage was achieved with platelet transfusions in the dog and rat,[1,6] and an "effective therapeutic regimen" for the hematopoietic syndrome in dogs (including antibiotics, fresh whole blood, and parenteral fluids) has been reported.[17] X and gamma irradiated monkeys have also been treated somewhat successfully with antibiotics.[2,14,18]

This report describes the effectiveness of a combination of readily available therapeutic measures in the treatment of beagles given mixed gamma-neutron doses known to cause grave injury to the hematopoietic system. Use of the beagle was based on its status as a well standardized and easily handled experimental animal which resembles man in many of its hematological parameters. Further, many studies of the beagle's responses to ionizing radiation have been made, including median lethality studies employing the same irradiation conditions as the present study.[10]

The therapeutic regimen chosen included prophylactic administration of antibiotics, biologicals, and other agents, followed by further treatment based upon clinical judgment. To minimize the development of antibiotic resistant microorganisms,

large doses and combinations of antibiotics were used. One group of beagles received blood element transfusions in addition to the antibiotics, biologicals, etc.

II. MATERIALS AND METHODS

Animals. Purebred beagles of both sexes, 2 to 3 years of age, were used. They had been immunized against distemper, infectious hepatitis, leptospirosis, and rabies. For at least 3 weeks prior to irradiation, the dogs were caged individually in temperature-controlled rooms, fed kibbled dog food and canned meat, and provided with fresh water ad libitum. During this period they were given a thorough physical examination and treated as indicated; at least two complete preirradiation hemograms were obtained for each dog. The dogs received no medication or unusual manipulation for 2 weeks prior to irradiation.

Group A, 32 dogs, were given postirradiation treatment including antibiotic, nutritional, and biological agents. Group B, 9 dogs, received the same postirradiation treatments as Group A plus leukocyte and platelet concentrates.

Radiation exposure. The AFRRI-TRIGA reactor and exposure facilities, described in detail elsewhere,[12] were used. The beagles were irradiated in Plexiglas boxes on wooden stands placed such that their midline was 400 cm from the reactor core center line. To achieve uniform (class A) irradiations, the exposure box was rotated 180 degrees about its vertical axis at the midpoint of each irradiation.

The midline tissue kerma rate, free-in-air, was determined with a 50 cm^3 tissue-equivalent ionization chamber. The ratio of the absorbed dose in the center of a beagle phantom to the tissue kerma, free-in-air, was determined with miniature tissue-equivalent ionization chambers. The product of these two quantities gave

the absorbed dose rate at the center line of the animal. All irradiations were at 17 rads/minute. The neutron to gamma ray tissue kerma ratio of the mixed gamma-neutron field was approximately 0.4.

Group A dogs, four at a time, were given 250- to 354-rad midline tissue doses (MTD). Group B dogs, singly or in pairs, were given 270 to 330 rads (MTD).

Observations and therapy. Irradiated beagles were examined one to three times a day depending upon their clinical condition. Blood specimens for complete hemograms were collected regularly to monitor the response to radiation and treatment.

The following treatments were used:

1. Furazolidone (Furoxone: Eaton) was orally administered (100 mg twice a day) to all dogs on postirradiation days 1 through 8.

2. Penicillin-dihydrostreptomycin (Combiotic: Pfizer) was administered intramuscularly (I.M.) to all dogs (400,000 U.S.P. units of penicillin and 0.5 g of dihydrostreptomycin daily) for 8, 6, or 4 days beginning on postirradiation days 4, 6, or 8, respectively. This treatment was repeated when indicated by the clinical course of each dog.

3. Oxytetracycline (Terramycin: Pfizer) was orally administered (250 mg three times a day) to all dogs for a period of 4 to 8 days beginning 12 days postirradiation. In some cases this treatment was repeated later in the course of illness.

4. Demethylchlortetracycline HCl (Declomycin: Lederle) was administered orally (300 mg twice a day) on the 1st day of fever and was continued until the temperature returned to normal or until death.

50- to 100-ml extract containing approximately 2×10^9 leukocytes and 3×10^{10} platelets. Turpentine in alcohol subcutaneously administered to donors 24 hours prior to collection of blood did not consistently increase the peripheral leukocyte count and was discontinued early in the study. Treatment of whole blood with high molecular weight dextran (190,000 mol wt) to cause rapid settlement of red blood cells did not consistently increase leukocyte yields and was similarly abandoned.

When inappetence occurred, canned meat, raw liver, and, in some cases, water were force fed.

Bacteriologic studies of blood from febrile dogs and of selected tissues from decedents were conducted. Decedents were necropsied as soon as possible after death.

The mortality data were analyzed by digital computer using a modified version of a United States Department of Agriculture probit analysis program to provide the maximum likelihood estimates described by Finney.[5]

III. RESULTS

Acute mortality data for Group A beagles are presented in Table I together with some clinical signs. All deaths occurred between postirradiation days 11 and 26. The $LD_{50/60}$ was calculated to be 276 rads (MTD), with a 95 percent confidence interval of 236 to 295 rads (Table II).

Clinical signs of radiation injury were generally less severe in the Group A dogs than described in previous median lethality studies;[10,13] however, vomiting incidence in the first few hours postirradiation was similar to that reported by Pitchford and Thorp.[13] Anorexia was common between the 8th and 28th days but

Table I. Clinical Signs and Mortality Data of Beagles Given Intensive Postirradiation Clinical Treatment (Group A)

Midline tissue dose (rads)	Percent mortality of dose group	Dog #	Days fever (>103°F)	First day leukocyte count <500 mm^{-3}	First day platelet count <10,000 mm^{-3}	Day of death
250	0	9*	13-19	13	-	survived
		10	24-25	-	18	survived
		11	19-24	20	-	survived
		12	19-25	-	15	survived
261	50	29	19-21	-	-	survived
		30	15-16, 23-25	23	9	26
		31	13-15	-	-	15
		32	22-24	-	-	survived
285	75	13†	12-15	15	11	16
		14‡	26	-	11	survived
		15	15-16	11	11	17
		16	13, 17	-	11	18
290	75	37	15	17	13	17
		38	19	17	13	survived
		39	13-15	10	10	16
		40	13-16	-	10	17
301	75	33	13-20	22	-	22
		34	23	15	-	survived
		35	12-14	-	-	15
		36	12-14	-	-	14
306	75	25	18-20	-	19	21
		26	13-16	-	-	17
		27	13-17	-	-	17
		28	23-26	-	13	survived
325	75	17§	15	15	11	16
		18	13-16	16	15	17
		19	17-22	22	11	survived
		20	16-18	-	11	19
354	100	21	16	15	-	17
		22	11-14	11	-	15
		23	10	11	-	11
		24**	11-13	11	11	13

* Edema of face and foot on day 18
† Gingival petechiation and edema of lips on day 11
‡ Gingival petechiation on day 11
§ Gingival petechiation and edema of nose on day 12
** Gingival petechiation and edema of foot on day 13

Table II. Probit Analysis Results of Group A Beagle Mortality Data

Percent mortality	Midline tissue dose (rads)	95 percent confidence limits (rads)
10	234	134-259
30	250	189-277
50	276	236-295
70	295	275-337
90	325	302-461
Slope of regression line - 17.9		

generally lasted only a few days except for the terminal anorexia of decedents. Petechial hemorrhage and edema were infrequent. Diarrhea was limited to 1 or 2 days preterminus in the decedents and no dehydration was noted.

Severe leukopenia (<500 cells/mm^3) and/or severe thrombopenia (<10,000 platelets/mm^3) were detected in about half of the subjects and were about equally distributed between survivors and decedents. However, only 2 of the 11 survivors had both severe leukopenia and thrombopenia while 8 of 21 decedents were so affected. Survivors suffered no more than 4 consecutive days of severe leukopenia while 17 of the decedents had severe leukopenia 6 or more consecutive days.

Severe neutropenia preceded onset of fever (103°F or over) by several days. Temperatures dramatically returned to normal on the day neutrophil counts clearly began to recover. Three subjects suffered fevers in the absence of severe leukopenia or neutropenia. Attempts to culture microorganisms from blood during febrile stages of illness were not successful.

Necropsy findings were consistent, with moderate to severe hemorrhagic lesions of the pleura, lungs, heart, and lymph glands but with minimal gastrointestinal hemorrhages. Edema and congestion of lungs were common and one subject had extensive bronchopneumonia. Bacterial cultures of organs and tissues of decedents were negative except for one subject in which E. coli was cultured from all tissues sampled. (This dog could not retain the oral medications after day 9 and thus received only injectable medication from then until death on day 14.)

Although prednisolone increased survival times and reduced mortality rates in irradiated monkeys,[14] it had no clear effect on the clinical course or survival of the beagles.

Group B mortality data are listed in Table III.

Table III. Mortality Data of Beagles Given Intensive Postirradiation Clinical Treatment Including Leukocyte and Platelet Concentrates (Group B)

Midline tissue dose (rads)	Dog #	Survival time (days)	Postirradiation days on which leukocyte and platelet concentrates were given
270	3	survived	7, 9, 12, 16, 17, 20
	1	survived	8, 10, 12, 18
	5*	36.7	16, 17
300	4†	42.3	15, 16, 17, 20, 21
	6	survived	none
330	2	survived	8, 11, 13, 17, 18
	7	14.7	6, 7, 8, 9, 10, 11, 15
	41‡	25.7	11, 14, 18
	8	survived	14, 15, 18

* Pulmonary edema on day 34

† Severe hemolytic anemia on day 35

‡ Transfusion of blood elements stopped on day 18 due to incompatibility of donors with recipient

The deaths of dogs numbers 5 and 4 were attributed to transfusion reactions. Blood typing to avoid selection of A positive dogs as donors was begun midway through the study and appeared to be of value.

The postirradiation days on which leukocyte-platelet concentrates were given are indicated in Table III; an increase in the circulating leukocytes and platelets was detectable on the day following administration (leukocyte counts increased as much as 400 mm^{-3} and platelets as much as 40,000 mm^{-3}). Four of the six Group B dogs suffering severe neutropenia more than 6 consecutive days survived and one of the two decedents died of a transfusion reaction.

The same association of fever with neutropenia found in Group A dogs occurred in Group B dogs. Bacteriological cultures of blood taken from Group B dogs during their periods of high fevers and lowest leukocyte levels were negative.

No alterations in hematologic parameters were detected in six unirradiated beagles given various combinations of Furoxone, Coly-Mycin M, Combiotic, Mycostatin, Declomycin, and Terramycin for 14 consecutive days at the doses used in treating Group A and Group B beagles.

IV. DISCUSSION

The $LD_{50/60}$ for Group A beagles (276 rads) was notably greater than that reported for untreated beagles irradiated under essentially the same conditions (218 rads).[10] The four highest doses in the previous study (235, 263, 284, and 292 rads) resulted in 100 percent mortality.[10] Similar doses in the current therapy study, 250, 261, 285, and 290 rads, caused 0, 50, 75, and 75 percent mortality, respectively. Table IV lists for review the mortality response from beagle studies at this

laboratory using essentially the same radiation parameters. Figure 1 illustrates the dose response regression lines from the two studies. The untreated beagles suffered diarrhea, dehydration, marked anorexia, excessive salivation, and other signs of acute illness and infection;[10] those signs of radiation illness were generally suppressed by treatment. Fever occurred but onset tended to be delayed by the treatment, and fever lasting several days did not necessarily forbode death as in untreated beagles.[10,13] Further, survival times of decedents appeared to be increased by treatment.

Bacteremia was not detected in antibiotic treated dogs. Coulter and Miller[3] reported similar results; their untreated irradiated dogs consistently had gram negative bacteremia, as did monkeys at this laboratory.[19] Pathologic findings in the current study included little evidence of infection, except for the one dog unable to retain oral medications after day 9.

Byron et al.[2] treated irradiated monkeys with antibiotics; 28 percent survived an LD_{99} dose of x rays (a dose that caused bacteremia and death in all untreated monkeys). Treatment of beagles in the current study was more effective (100 percent survived an LD_{98} dose); however, antibiotic therapy was more extensive and additional supportive measures were employed.

Three beagles receiving platelet and leukocyte concentrates in addition to the therapy given Group A dogs experienced transfusion incompatibility reactions (subsequently avoided by selection of canine A negative donors). If these three dogs are eliminated, then five of six Group B dogs survived doses far exceeding the LD_{99} (254 rads). Sorensen et al.[17] achieved only 80 percent survival of dogs by antibiotic treatment and blood transfusions following an LD_{90} dose of x rays.

Table IV. Mortality Response of Beagles with and without Postirradiation Treatment

Untreated*		Antibiotics (Group A)		Antibiotics plus blood elements (Group B)	
Midline tissue dose (rads)	Mortality (percent)	Midline tissue dose (rads)	Mortality (percent)	Midline tissue dose (rads)	Mortality (percent)
235	100	250	0		
263	100	261	50	270	33
284	100	285	75		
292	100	290	75		
		306	75	300	50
		325	75	330	50

* George et al.[10]

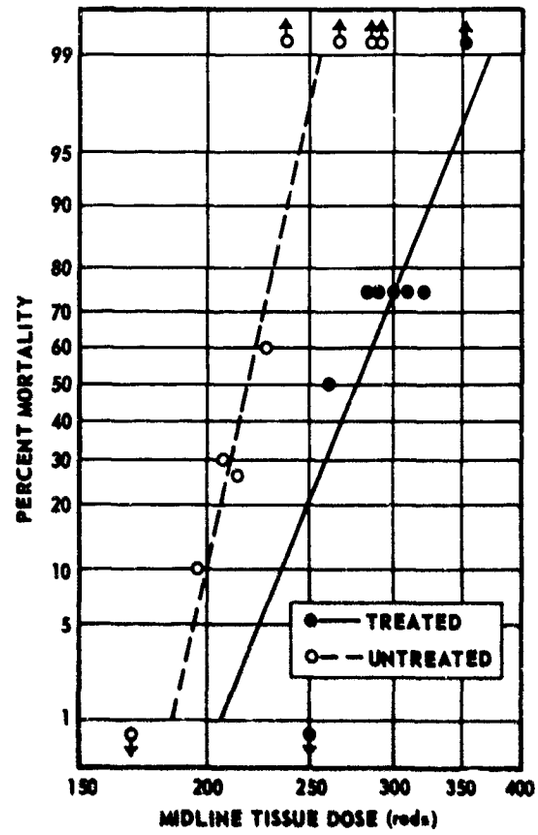

Figure 1. Dose response regression lines for beagles bilaterally exposed to mixed gamma-neutron radiation

V. SUMMARY

Readily available antibiotics and supportive treatment were used successfully to markedly decrease mortality following radiation doses exceeding the LD_{99} for untreated beagles. Prophylactic use of antibiotics, although somewhat controversial, minimized signs of infection in both survivors and decedents. Arguments that resistant organisms may develop or that hematopoiesis may be depressed by such measures were not supported by the findings of this study. The microorganisms reported to cause fatal post-irradiation bacteremia in monkeys[19] and dogs[3] are gram negative enteric bacteria which presumably crossed the intestinal barrier and multiplied; the rapid course of such infections does not allow sufficient time to await appropriate clinical signs before initiation of treatment; therefore, early administration of enteric and systemic antibiotics in massive doses appears justified in treating injury from life threatening doses of radiation.

Antibiotic therapy plus leukocyte and platelet administration showed remarkable promise for enhancing survival of lethally irradiated beagles. However, such transfusion procedures are complicated by the many donors necessary to support each patient, and the incompatibility reactions which must be avoided through careful typing and selection of donors.

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